Effect of Ivermectin on *Anopheles gambiae* Mosquitoes Fed on Humans: The Potential of Oral Insecticides in Malaria Control

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Anopheles gambiae **mosquitoes were fed on 25 volunteers randomized to receive ivermectin or nothing. In mosquitoes feeding on volunteers given ivermectin the previous day, mean survival was 2.3 days, compared with 5.5 days in the control group ($P < .001$, by log-lank test). Mosquito mortality was 73%, 84%, and 89% on days 2, 3, and 4 in the ivermectin group. In mosquitoes fed 14 days after treatment, no difference was found between groups. Ivermectin is safe and has significant short-term insecticidal properties. A systemic insecticide taken by humans could help to control malaria in areas where mosquitoes are exophagic or exophilic and drug resistance is an urgent threat.**

There remain formidable technical challenges in reducing deaths due to malaria to zero and the eventual elimination of the disease. Two major challenges are outdoor biting by mosquitoes and the emergence of drug resistance.

Exophilic and exophagic (outdoor biting) mosquitoes are a problem for malaria control in Asia and South America but are less important in Africa [1, 2]. In places where most transmission is outdoors, residual spraying and, probably, insecticide-treated bed nets have reduced effect. This complicates attempts to control malaria in areas of emerging drug resistance, such as Cambodia [3]. In parts of Asia and Latin America, the proportion of malaria transmission occurring outdoors increased following large-scale deployment of residual spraying. There are limited options for vector control of exophilic mosquitoes, especially where forest transmission is common and larval control impractical (as in Southeast Asia). The emergence of antimalarial drug resistance is another stumbling block to malaria control and depends on mosquitoes transmitting malaria from people who have taken antimalarial drugs.

It has been known since development of the Macdonald-Ross equation that the greatest effect on malaria transmission is produced by decreasing the longevity of mosquitoes that bite humans. An intervention that could reduce the survival of mosquitoes which bite humans outdoors would be an attractive prospect.

Ivermectin has a wide antiparasitic activity with long veterinary use [4]. When ivermectin's activity against *Onchocerca volvulus* was discovered, it was licensed for human use and was used in mass drug administration programs to control river blindness; it was administered to >80 million adults and children. The drug has proven to be safe. Doses up to 10 times the approved limit are well tolerated by healthy volunteers [5]. Adverse reactions are few and usually mild [6, 7]. Ivermectin is also used against other human parasites, including strongyloides and scabies.

Animal and in vitro experiments have shown that ivermectin has lethal effects on blood-sucking insects when these are fed on treated blood samples. It increases mortality and reduces the fertility of Tsetse flies, triatomine bugs, ticks, and sandflies. Experiments in animals and in vitro have shown that ivermectin readily kills adult mosquitoes, including anophelines [8, 9]. In human trials of mass drug administration for onchocerciasis and filariasis, the effect of ivermectin on mosquitoes seems to be maintained in the field [10, 11]. These insecticidal properties of ivermectin have led some to suggest that this drug could have a role in the control of arthropod vector–borne diseases. Given this indirect evidence we therefore set out to test directly the effect of ivermectin on *Anopheles gambiae,* the major vector of malaria in Africa, in a controlled study.

Methods. Volunteers were recruited at the London School of Hygiene and Tropical Medicine (LSHTM), following receipt of informed consent. Exclusion criteria were as follows: age <18 or >50 years; recent visit to a malarious area; having lived for >3 months in an area endemic for Loa loa; and in female volunteers, pregnancy, breast feeding, or last menstrual period >28 days before the study.

Volunteers were randomly assigned to receive a single oral dose of 200 μg/kg of ivermectin (Merck) or no drug, as a control. The sequence of randomization was generated in Stata

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Table 1. Mosquito Mortality at Selected Day.

Group, variable	Start	Day 1	Day 2	Day 3	Day 4	Day 9
Ivermectin						
No of deaths	267	84	110	29	13	19
Daily mortality, % of mosquitoes dead	…	31	60	39	29	61
Cumulative mortality, no (%) of mosquitoes dead	…	84 (31)	194 (73)	223 (84)	236 (88)	255 (96)
Control						
No of deaths	250	48	30	16	16	69
Daily mortality, % of mosquitoes dead	…	19	14	9	10	49
Cumulative mortality, no (%) of mosquitoes dead	…	48 (19)	78 (31)	94 (38)	110 (44)	179 (72)

8 (StataCorp) with blocks of 4 random digits. Allocation was performed by means of sealed opaque envelopes given to the volunteers. The study was nonblinded to patients and physicians, but laboratory staff and those assessing effects on mosquitoes were blind to treatment allocation.

Mosquitoes were *A. gambiae* from the G3 colony, originally from the Gambia. Mosquitoes were bred from the colony eggs with use of standard methodology and were maintained at 26°C–27°C with a 12:12 photoperiod. When >1 larvae bowl was used, the resulting adults were mixed in a reserve cage to reduce confounding from the use of different batches. Pupae were introduced in the reserve cage regularly in an attempt to keep the age of mosquitoes stable.

Volunteers attended the Hospital for Tropical Diseases in London. The attending physician opened the envelopes and supervised the administration of the drug when indicated. The following day, volunteers attended the insectary of LSHTM, where 22 female mosquitoes were selected by holding a hand close to the cage and using a mouth aspirator to pick hungry females approaching to bite. While in the aspirator, antennae were inspected to check for the presence of male mosquitoes, which were reintroduced to the reserve cage. Females were transferred to prenumbered feeding cups and applied to the forearm of each volunteer for 10–12 min.

Mosquitoes were then transferred to individually labelled cages and maintained on 10% glucose solution. Mortality was recorded daily at the same time for 12 days. Six days after the human blood meal, an additional blood meal was offered from horse blood in an artificial feeding device. Egg bowls were introduced in the cages 48–72 h after blood feeding to allow for normal egg laying. Fourteen days after the initial ivermectin treatment, the feeding process was repeated with fresh mosquitoes to test the delayed effect of ivermectin.

The unit of intervention was clusters of 22 female *A. gambiae* mosquitoes. The primary outcome was geometric mean mortality of mosquitoes with an emphasis on day 3 after feeding (the approximate duration of the gonotrophic cycle at 30°C) and day 9 (the minimum duration of the sporogonic cycle).

Sample size calculations demonstrated 12 clusters in each arm were needed to detect a difference in mortality between the control and the intervention groups of 25% versus 50% at the 5% level of significance, with a power of 80% and with use of clusters of 22 mosquitoes and allowing for 10% nonfeeders. The design effect was calculated as 4.25 with use of an intercluster variation coefficient of 0.45. The daily mortality rates for each intervention group were pooled. Kaplan-Meier survival curves were constructed using Microsoft Excel and Addinsoft XLSTAT. Comparisons were made using the log-rank test. Quoted means are geometric. The χ^2 values were corrected for the cluster effect [12]. Hazard ratios were calculated using Cox's regression.

Ethical approval was obtained from the Ethics Committee of LSHTM. Written informed consent was obtained from all volunteers.

Results. Of 25 volunteers recruited June–July 2008, 13 were randomized to receive ivermectin. For day 1, mosquitoes were included in 25 clusters of size 22. The second stage involved 24 clusters.

Baseline characteristics of volunteer groups were similar in weight and age, with more men in the control group. All the volunteers attended the first feeding, with 1 volunteer lost to follow-up for the day 14 feeding.

For the day 1 feedings, mean duration of survival of the mosquitoes in the ivermectin group was 2.38 days (95% confidence interval [CI], 1.52–3.24 days), whereas the mean duration of survival in the control group was 5.52 days (95% CI, 4.65–6.40 days; $P < .001$, by log-rank test). Cumulative mortality of mosquitoes in the ivermectin group was calculated as 73%, 84%, and 89% at days 2, 3, and 4, respectively, compared with 32%, 38%, and 44% in the control group. The difference decreased on subsequent days. At day 9 after feeding, the earliest time at which a mosquito becomes infectious, the mortality in the ivermectin group was significantly higher than in the control group (96% vs 73%) (Table 1). Cox's proportional hazard ratio for survival to day 12 was 2.22 (95% CI, 1.83–2.7). Figure 1 shows the Kaplan-Meier survival curve.

When the blood meal is taken 14 days after the treatment, the difference seen in the mortality at days 2, 3, or 9 after feeding is no longer apparent. The mean duration of survival in the ivermectin group was 6.83 days (95% CI, 6.09–7.57 days),

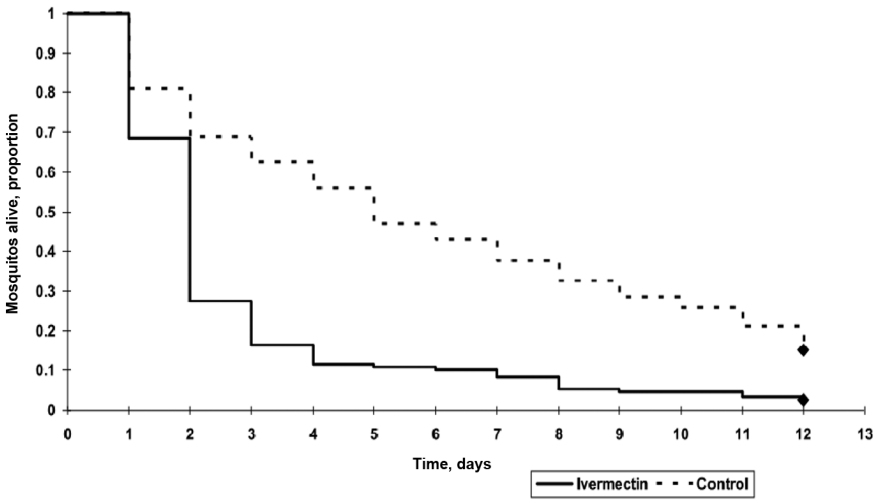

Figure 1. Survival distribution function of mosquitoes fed on volunteers 1 day after treatment.

and the mean duration of survival in the control group was 6.88 days (95% CI, 6.09–7.67 days; $P = .51$, by log-rank test). The Cox's proportional hazard ratio was 1.07 (95% CI, 0.87–1.31).

Discussion. This study of ivermectin in a dose appropriate for human use led to a significant reduction in *A. gambiae* survival when they fed on treated humans, compared with controls, 1 day after treatment but had no obvious impact on survival by mosquitoes fed 2 weeks later after treatment. This provides confirmation that a safe systemic insecticide given to humans can lead to a significant impact on malaria vector longevity. The effect was seen early, such that even if the mosquitoes were infectious, they would have a high chance of dying before the next blood meal, and if the mosquitoes were infected in the bite where they ingested ivermectin, they would have a low chance of surviving to become infectious 9–10 days later. A nonmeasured proportion of mosquitoes in the ivermectin group that were still alive were noted to show lack of movement coordination, lethargy, inability to fly, and paralysis, which means that the overall effect on survival may be even greater in the wild.

That ivermectin is insecticidal to this major malaria vector is consistent with its effects on other arthropods, including other mosquito species, from in vitro and animal work and with the indirect data from studies of the effect of mass drug administration. The literature varies on the longevity of the insecticidal effect, with some finding a prolonged effect [10]. The effects of ivermectin on blood-sucking insects and parasites may be only loosely related to plasma concentration, possibly because of metabolites with longer half-lives [11, 12]. Data on the pharmacokinetics of ivermectin in humans vary. After oral administration, ivermectin reaches peak plasma levels at about 4 h [13], and the effects observed on mosquito mortality might have been greater if the blood meals had taken place on the same day of the treatment and not 24 h later. Half-life has been variably calculated to be 12–28 hours [14].

The potential for a systemic insecticide has been realized for some time [15]. There are at least 4 possible uses for an effective nontoxic insecticide with activity against malaria vectors, although for 3 of these, oral ivermectin would not be appropriate because of the short duration of activity this study demonstrates. These 3 uses are (1) mass drug administration as part of a control strategy in areas where mosquitoes are exophilic; (2) treatment of domestic animals in areas where important vectors take some of their blood meals from animals; (3) administration as an altruistic prophylactic to individuals who are likely to import malaria into communities, such as gem miners or loggers in South America and Southeast Asia. All of these would aim to reduce transmission overall. This could be extended with a longer acting drug or perhaps with long-acting slow-release technologies. If used in this way, systemic insecticides would have an additional impact on other ectoparasite-transmitted diseases and, in the case of ivermectin, on the prevalence of intestinal helminths, which would increase its acceptance among the apparently healthy population.

The fourth use would be to combine a systemic insecticide with antimalarials to reduce the spread of antimalarial drug resistance. There is a limited range of situations where this would be appropriate, although the start of resistance to a major drug class in an area where mosquitoes are exophilic is potentially one such situation, and these conditions are met in Cambodia at present. Public health benefits would have to be weighed against the (small) risk of adverse effects of the drug.

Data on longevity of mosquitoes in an insectary cannot automatically be extrapolated to wild mosquito populations. This could either lead to an underestimation of the effect (if the

oddly moving mosquitoes died in the wild) or an overestimate. Only 1 malaria vector was tested; it is possible that the effect would be different in others. However, the data are consistent with other mosquito and insect species; thus, it seems unlikely that the effect is restricted to this species. Before plans for any public health intervention could be tested, it would be important to test the effect of ivermectin on days 2–14 after ingestion.

This study demonstrates that ivermectin, a drug with a good safety profile, can have a major impact on the longevity of an important malaria vector and that it acts rapidly enough to have practical implications for control but is only for a short time after the drug has been taken. Systemic insecticides should be investigated for those areas where exophagic mosquitoes constitute a significant proportion of the transmission of malaria.

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